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Melatonin Suppression by Light in Humans Is Maximal When the Nasal Part of the Retina Is Illuminated

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Abstract This study investigated whether sensitivity of the nocturnal melatonin suppression response to light depends on the area of the retina exposed. The reason to suspect uneven spatial sensitivity distribution stems from animal work that revealed that retinal ganglion cells projecting to the suprachiasmatic nuclei (SCN) are unequally distributed in several species of mammals. Four distinct areas of the retinas of 8 volunteers were selectively exposed to 500 lux between 1:30 a.m. and 3:30 a.m. Saliva samples were taken before, during, and after light exposure in 1-h intervals. A significant difference in sensitivity was found between exposure of the lateral and nasal parts of the retinas, showing that melatonin suppression is maximal on exposure of the nasal part of the retina. The results imply that artificial manipulation of the circadian pacemaker to alleviate jet lag, to improve alertness in shift workers, and possibly to treat patients suffering from seasonal affective disorder should encompass light exposure of the nasal retina.

Key words melatonin, retina, sensitivity, light, human, circadian, photoreceptor

INTRODUCTION

The retina is the primary light-sensitive organ conveying information on the natural light-dark (LD) cycle that entrains mammalian circadian systems. Although the nature of the photoreceptors involved in the process is unknown (Provencio and Foster, 1995), neuroanatomical information on the spatial distribution of ganglion cells projecting to the circadian pacemaker in the suprachiasmatic nuclei (SCN) suggests that not the whole retina is involved in this process. In rats and hamsters, the ganglion cells projecting to the SCN are distributed evenly throughout both retinas (Pickard, 1980, 1982). In sheep, these cells are exclusively present in the upper halves of the retinas (Cooper et al., 1993). Only a single primate has been studied in this respect. This was a macaque, where the nasal parts of the retinas were much more densely packed with ganglion cells projecting to the SCN than were the lateral parts (Cooper, personal communication). For humans, no information about the connections between retina and SCN is available, although we know that the exposure to light of both eyes leads to larger melatonin suppression than does exposure of one eye (Brainard et al., 1997). There is a growing interest in resetting the human circadian pacemaker by artificial light, for example, for adjusting circadian phase after transmeridian flights, for improving alertness during shift work, and for treating seasonal affective disorder (SAD). For the optimization of technology in such light treatments, it would be important to know from which areas of the retinas the human circadian system receives its inputs.

Retrograde staining studies similar to those in animals cannot be performed in humans, but functional studies are feasible. We have exploited the fact that melatonin production in the pineal gland is under control of the SCN. Lesions of the SCN of rodents and

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sheep abolish the rhythmicity of melatonin secretion (Moore, 1993; Moore and Klein, 1974; Tessonneaud et al., 1995) but allow a basal melatonin synthesis to continue. Such lesions prevent the melatonin suppression response to light (Hastings and Herbert, 1986; Kalsbeek et al., 1996). Hence, it is possible to obtain functional information about the pathways from retina to SCN by investigating melatonin suppression in response to light. Under conditions of normal circadian entrainment, melatonin is secreted during the night in humans, as in other mammals. Circulating levels begin to rise in the evening, reach maximal values in the middle of the night (2:00-4:00 a.m.), and then progressively decrease toward minimal values in the morning. Circadian profiles of melatonin are sufficiently intraindividually consistent to use melatonin as an indicator for circadian light sensitivity (Lewy et al., 1997). In the present study, we tested the extent of melatonin suppression on exposure to light of four distinct retinal areas: upper and lower nasal and upper and lower temporal. We observed that the suppression of nocturnal melatonin by light is most effective on exposure of both the upper and lower nasal parts of the retina. This suggests that the circadian pacemaker in the SCN is mainly sensitive to light in the lateral visual fields.

MATERIALS AND METHODS

Subjects

A total of 8 healthy subjects were recruited through advertisement (4 females, ages 21-26 years; 4 males, ages 21-31 years) and gave informed consent to participate in this study. Subjects were nonsmokers and were asked to maintain their own preferred sleep-wake schedules for at least 3 days preceding each experimental night. The study was carried out in the "time-signal free unit" of the Biological Centre of the University of Groningen in September 1997. All subjects were studied overnight on four occasions at 1-week intervals.

To avoid interference with the melatonin assays, subjects were instructed not to ingest any coffee on or during the evening of the experiment because caffeine will suppress nocturnal melatonin levels (Wright et al., 1997). Subjects were not allowed to use lipstick or to eat bananas on the experimental evening. These substances may interact with the radioimmunoassay (RIA) procedure to measure melatonin concentrations

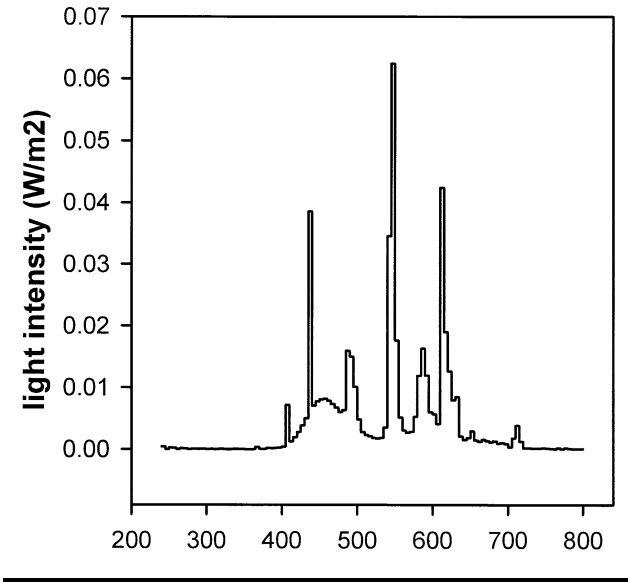

Figure 1. Spectrum of the applied light source measured with a cosine sensor, positioned in the direction of gaze of the subjects.

(Gordijn, Jansen, Medema, Flengte, and Beersma, submitted; Gordijn, personal communication). From 10:00 p.m. to 4:30 a.m., subjects were allowed to drink only water, but not within a half-hour before a saliva sample was taken, to avoid dilution of the sample. Before and during saliva collection, subjects were seated in comfortable chairs to avoid postural changes to affect melatonin concentration (Deacon and Arendt, 1994; Morris et al., 1990).

Light Treatment

From 10:00 p.m. to 1:30 a.m. and from 3:30 a.m. to 4:30 a.m., subjects were exposed to dim light (< 10 lux). From 1:30 a.m. to 3:30 a.m., subjects were seated in comfortable chairs and instructed to watch a video screen at 3.3 m continuously. Two light boxes were positioned at an angle of 30° left and right relative to the direction of gaze of the subjects. The center of the light source was placed either at 23° above or at 23° below the horizontal plane. The light source was a box containing eight fluorescent tubes (SunSquare, Sunbox, Gaithersburg, MD). Subjects were exposed to 500 lux light intensity. Light intensity was measured at the level of the cornea with a cosine lux meter (Lutron LX-101, Taiwan). The light spectrum of the lamps was measured with a Macam photometric meter (SR9910-PC, Photometrics, Livingstone, UK) with a cosine sensor (Fig. 1).

To achieve symmetric illumination of the same areas of each retina, subjects wore one of two different

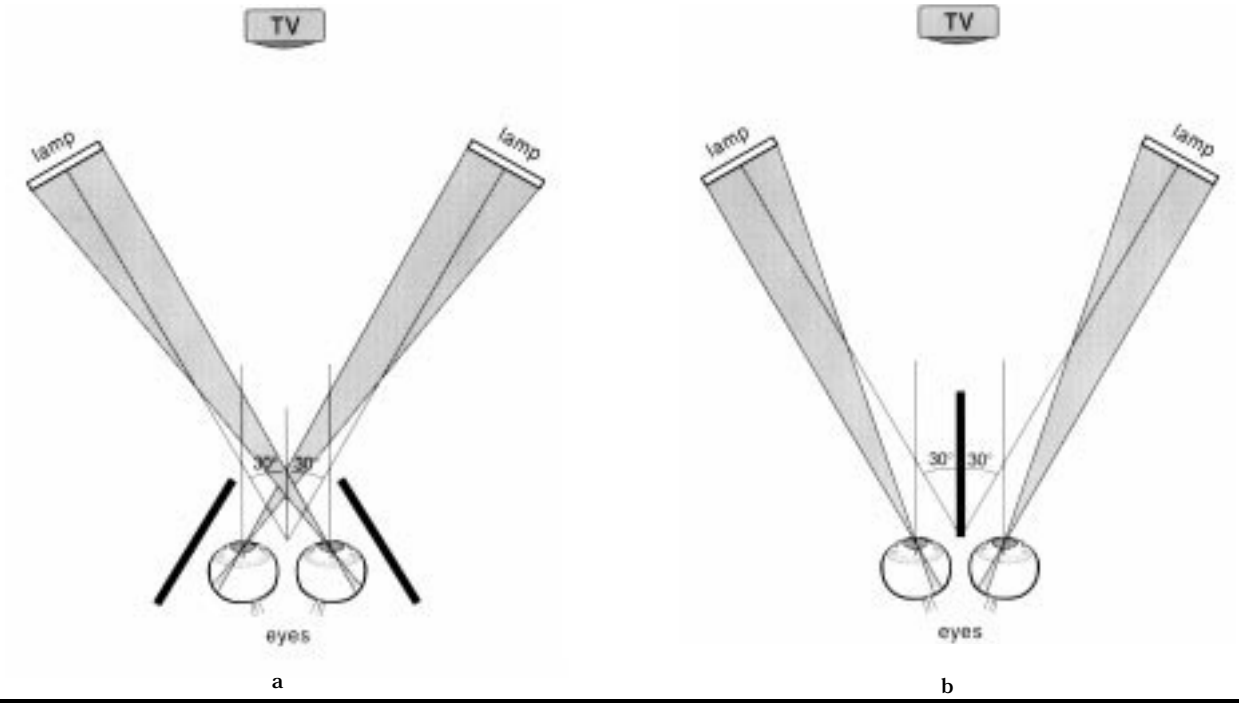

Figure 2. Positions of helmet and light boxes (a) while exposing the lateral area of the retina and (b) while exposing the nasal area of the retina.

helmets during illumination. These helmets were equipped with shields to cause light from each light box to reach only one retina. One helmet had two black cardboard covers at the outside, positioned in such a way that the light of the right lamp entered only the left eye and the light of the left lamp entered only the right eye (Fig. 2a). The other helmet was equipped with a piece of black cloth above and along the nose and pointing straight forward; this helmet prevented light from the lamp positioned to the right of the subject from entering the left eye and prevented light from the left lamp from entering the right eye (Fig. 2b). These two helmets were used with lamps that were placed both 23° above the horizontal plane and 23° below it. In this way, four conditions were created. In Condition 1, the lower lateral parts of both retinas were illuminated. In Condition 2, only the upper lateral parts of both retinas were illuminated. In Condition 3, only the lower nasal parts of both retinas were illuminated. In Condition 4, only the upper nasal parts of both retinas were illuminated. Subjects were exposed to the four conditions in quasi-random order in weekly trials.

When cardboard covers on the outside of the helmets were used (Conditions 1 and 2), the lamps were covered 27% on the outside with cardboard to minimize interindividual variation in illumination area of the retina, caused by different shapes of head and nose. In all conditions, the same light intensity was generated at the eye level by adjusting the distance between eyes and the light source. This distance was 1.90 m in Conditions 3 and 4 and was 1.68 m in Conditions 1 and 2. Thus, the light intensity was 500 lux, measured at eye level, in all conditions. The retinal area illuminated was calculated in steradians as a/r^2, in which a is the area of the light source and r is the distance from the light source to the eye. Introducing this to the four conditions used in this study, the solid angle of Conditions 1 and 2 was 0.075 steradians and in Conditions 3 and 4 was 0.081 steradians.

Melatonin Measurements

Saliva samples of at least 1 cc were collected during the night at 12:45, 1:30, 2:30, 3:30, and 4:30 a.m. The first two samples and the final one were taken in dim light conditions; the other two were taken in bright light. Saliva samples were collected in Salivette tubes (Sarstedt, Etten-Leur, The Netherlands) and stored at -20°C until assayed for melatonin.

Analysis was done by RIA in the biochemistry laboratory of the Academic Hospital of Groningen. Saliva samples were thawed and centrifuged to remove particulate matter. A rapid direct saliva RIA was done using ^{125}I-2-iodomelatonin as radioligand and rabbit

anti-melatonin (Stockgrand, Guildford, Surrey, UK) as antiserum (English et al., 1993). The detection limit of the procedure was 4 pg/ml. The intraassay standard deviation was 2.7 pg/ml, and the interassay standard deviation was 2.8 pg/ml.

RESULTS

Saliva melatonin concentrations preceding light treatment (i.e., at 12:45 a.m. and 1:30 a.m.) varied from 5 to 86 pg/ml and were not significantly different between conditions, analysis of variance (ANOVA), $F(3, 60) = 0.07$, n.s., whereas there were significant between-subject variations, ANOVA, $F(7, 56) = 118.76$, $p < .001$. For each subject and each condition, we took the average of these two values and expressed all values as a fraction of this average. These relative values were log-transformed. Figure 3 presents the mean log relative saliva melatonin concentrations as a function of time.

Overall, the melatonin content was suppressed under the influence of 500 lux light intensity. Maximum effects were observed at the end of the 2-h light exposure (3:30 a.m.). At this time, illumination of the lateral parts of the retina suppressed the melatonin concentration on average by 33% (lower lateral) and 22% (upper lateral). Exposure of the nasal parts of the retina yielded a suppression of melatonin secretion of 63% (lower nasal) and 59% (upper nasal). At 4:30 a.m., when lights had been switched off for 1 h, melatonin levels had nearly returned to the levels before light exposure. ANOVA applied to the log-transformed relative data obtained at the end of light exposure revealed a significant contribution to the explained variance of four conditions, $F(3, 28) = 3.29$, $p = .041$, and between subjects, $F(7, 24) = 3.48$, $p = .012$. Post hoc analysis revealed that there was a significant difference between exposure of the nasal and lateral areas of the retina, $F(1, 14) = 7.28$, $p = .031$. There was no significant difference in melatonin suppression when exposure of upper and lower parts of the retina were compared, $F(1, 14) = 0.44$, $p = .530$.

DISCUSSION

This study shows that the extent of nocturnal melatonin suppression by light of 500 lux intensity depends on the area of the retina that is exposed. Melatonin suppression is significantly larger on exposure of the nasal part of the retina, that is, when the light boxes are presented in the lateral visual field of each eye. The

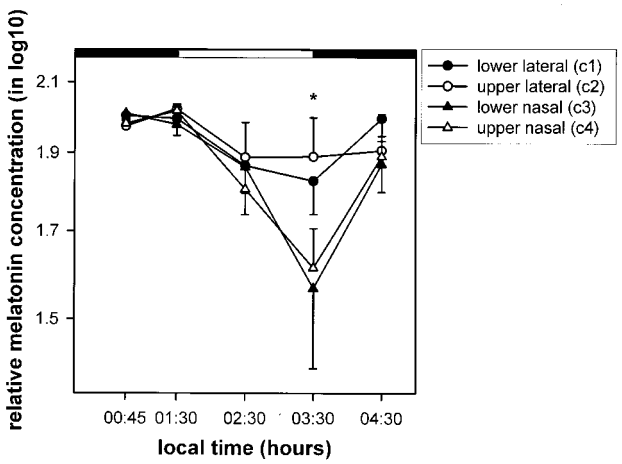

Figure 3. Saliva melatonin concentrations as a function of time, expressed as a fraction of the average of the first two values of each condition, which were obtained in dim light prior to light exposure. * denotes a significant difference between conditions.

retinal areas illuminated in the nasal area slightly exceeded (by 6.6%) those illuminated in the lateral retina due to the procedure used to maintain the light intensity at exactly 500 lux. In spite of this small difference, melatonin suppression was more than doubled with nasal illumination. These large differences in melatonin responses were observed in spite of the facts that the direction of gaze of the subjects was not strictly fixated and intraocular light scatter could not be avoided. In addition, reflections from the walls of the room might have interfered. Each of these factors could, at most, have reduced the differences between conditions. Because it can be expected that the reduction of the differences is larger with higher light intensity of the source, we have chosen to use 500 lux light intensity— sufficient to see significant melatonin suppression and yet not so intense as to bleach the entire retina, irrespective the position of the light source. Apart from this, it has been reported that pupil responses are larger on exposure of the nasal part of the retina (Kardon et al., 1991). Although we did not measure pupil diameter in our experiments, it can only be expected that differential pupil responses would have further reduced the differences between conditions. In conclusion, each of these aspects suggests that the actual differential sensitivity of the retinal areas is underestimated in our experiment.

In rodents, the SCN are necessary for melatonin suppression by light to occur (Kalsbeek et al., 1996). If this holds for humans as well, then our finding implies that the human circadian pacemaker is differentially

sensitive to light depending on the retinal area exposed. Thus, it is to be expected that resetting the circadian pacemaker is most effective with light exposure in the lateral visual field. Phase correction after transmeridian travel or for night shift workers should be performed by such exposure of the nasal part of the retina. A coincidental advantage of this finding is that exposure of the foveal area of the retina is not necessary for phase-resetting purposes (Adler et al., 1992). Therefore, in most situations, the light applied for shifting phase does not need to interfere with other visual tasks.

The results also are important in the context of research on SAD. It often is thought, although not proven, that SAD is caused by some disturbance of the circadian system (Rosenthal et al., 1984) and that bright light is effective as a treatment through correction of this disturbance (Lewy et al., 1987). The difficulty to prove or disprove the role of the circadian pacemaker in SAD is partly due to the fact that light treatment is visible to the patient and no perfect placebo control treatment has been found. If the pacemaker is involved in the pathogenesis of SAD, then it should now be possible, by varying the position of the light stimulus, to modify the impact of the light on the pacemaker without change in conscious intensity perception. This could be a way in which to further unravel the role of the circadian system in light treatment of SAD.

The apparent gradient in spatial sensitivity of the human circadian pacemaker resembles the retinal distribution of ganglion cells reported in the macaque; in this species, the nasal parts of the retinas showed a much higher density of ganglion cells projecting to the SCN than did the lateral parts (Cooper, personal communication). In sheep, a higher density of such ganglion cells was found in the upper halves of the retinas (Cooper et al., 1993). By contrast, rats, mice, and hamsters, whose eyes have more axial divergence, have a more homogeneous distribution of such ganglion cells (Balkema and Drager, 1990; Pickard, 1980, 1982, 1985; Moore et al., 1995). These interspecies differences bear some resemblance with anatomical differences within the optic chiasm. The degree of crossing over of fibers of the visual perception pathway is associated with the angle between the ocular axes. In lateral-eyed animals, they cross completely in the optic chiasm, whereas in primates with strong binocular vision, only half of the fibers, in particular those from the nasal part of the retina, cross (McIlwain, 1996; Moore, 1993; Pickard, 1985). It is possible that the SCN receive only inputs from the bundle of fibers crossing over in the optic chiasm.

A recent study by Campbell and Murphy (1998) suggested the existence of extraocular pathways by which the SCN obtain information about light and darkness. Those authors demonstrated phase shifts of the human body temperature rhythm and of the melatonin secretion rhythm in response to light applied to the skin. It was suggested that some humoral factor in the blood could be involved in the transmission of the LD signal to the SCN. In the context of this hypothesis, it is conceivable that differences in blood flow and blood vessel density in various parts of the retina would cause the observed differences in sensitivity of melatonin suppression by light. However, no obvious differences in blood flow and blood vessel density are reported between nasal and lateral parts of the retinas (Wise et al., 1971).

Adler et al. (1992) and Lasko et al. (1999 [this issue]) have applied techniques similar to ours to compare the responsiveness of melatonin suppression by light at different exposure sites of the retina. Melatonin levels did not differ after central and peripheral illumination (Adler et al., 1992), whereas melatonin secretion was significantly suppressed after exposure of the upper visual field compared to levels after exposure of the lower visual field (Lasko et al., 1999 [this issue]). The apparent inconsistency between the present results and those of Adler et al. (1992) may be explained on the basis of a methodological difference. The lateral illumination condition of Adler et al.'s experiment was applied without using a mask to avoid contralateral eye exposure. So, while the nasal part of the ipsilateral eye was illuminated, the lateral part of the contralateral eye was illuminated simultaneously. Thus, the net result for lateral illumination might, in fact, have been intermediate between a strong effect from nasal retina exposure and a weak effect from lateral exposure, just like central exposure is expected to achieve. Our results do not confirm those of Lasko et al. (1999 [this issue]), as we found no difference between lower and upper illumination at either the lateral side or the nasal side of the retinas. A more fine-grained spatial analysis of different retinal segments will be needed to eventually delineate the area of maximal sensitivity. The combined results of Lasko et al. (1999 [this issue]) and our study might suggest a declining gradient in sensitivity from lower nasal to upper lateral segments. In the absence of more detailed information, it appears that light stimuli in the lateral visual field induce the largest effects on the melatonin secretion. We hypothesize that

this is due to a gradient in the presence of ganglion cells in the retina projecting to the SCN.

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REFERENCES

Adler JS, Kripke DF, Loving RT, and Berga SL (1992) Peripheral vision suppression of melatonin. J Pineal Res 12:49-52.

Balkema GW and Drager UC (1990) Origins of uncrossed retinofugal projections in normal and hypopigmented mice. Vis Neurosci 4:595-604.

Brainard GC, Rollag MD, and Hanifin JP (1997) Photic regulation of melatonin in humans: Ocular and neural signal transduction. J Biol Rhythms 12:537-546.

Campbell SS and Murphy PJ (1998) Extraocular circadian phototransduction in humans. Science 279:396-399.

Cooper HM, Tessonneaud A, Caldani M, Locatelli A, Richard S, and Viguier Martinez M (1993) Morphology and distribution of retinal ganglion cells (RGC) projecting to the suprachiasmatic nucleus in the sheep. Am Soc Neurosci Abstr 19:1704.

Deacon S and Arendt J (1994) Posture influences melatonin concentrations in plasma and saliva in humans. Neurosci Lett 167:191-194.

English J, Middleton BA, Arendt J, and Wirz-Justice A (1993) Rapid direct measurement of melatonin in saliva using an iodinated tracer and solid phase second antibody. Ann Clin Biochem 30:415-416.

Hastings MH and Herbert J (1986) Neurotoxic lesions of the paraventriculo-spinal projection block the nocturnal rise in pineal melatonin synthesis in the Syrian hamster. Neurosci Lett 69:1-6.

Kalsbeek A, Drijfhout WJ, Westerink BHC, Van Heerikhuize JJ, Van der Woude TP, Van der Vliet J, and Buijs RM (1996) GABA receptors in the region of the dorsomedial hypothalamus of rats are implicated in the control of melatonin and corticosterone release. Neuroendocrinology 63:69-78.

Kardon RH, Kirkali PA, and Thompson HS (1991) Automated pupil perimetry: Pupil field mapping in patients and normal subjects. Ophthalmology 98:485-496.

Lasko TA, Kripke DF, and Elliott JA (1999) Melatonin suppression by illumination of upper and lower visual fields. J Biol Rhythms 14:122-125.

Lewy AJ, Cutler NL, Hughes RJ, and Sack RL (1997) Week-to-week reproducibility of 500 lux light suppression of melatonin (LSM) in humans. Sleep Res 26:732.

Lewy AJ, Sack RL, Miller LS, and Hoban TM (1987) Antidepressant and circadian phase-shifting effects of light. Science 235:352-354.

McIlwain JT (1996) *An Introduction to the Biology of Vision*, Cambridge University Press, Cambridge, UK.

Moore RY (1993) Organization of the primate circadian system. J Biol Rhythms 8(Suppl.):S3-S9.

Moore RY and Klein DC (1974) Visual pathways and the central neural control of a circadian rhythm in pineal serotonin *N*-acetyltransferase activity. Brain Res 71:17-33.

Moore RY, Speh JC, and Card JP (1995) The retinohypothalamic tract originates from a distinct subset of retinal ganglion cells. J Comp Neurol 352:351-366.

Morris M, Lack L, and Barrett J (1990) The effect of sleep/wake state on nocturnal melatonin excretion. J Pineal Res 9:133-138.

Pickard GE (1980) Morphological characteristics of retinal ganglion cells projecting to the suprachiasmatic nucleus: A horseradish peroxidase study. Brain Res 183:458-465.

Pickard GE (1982) The afferent connections of the suprachiasmatic nucleus of the golden hamster with emphasis on the retinohypothalamic projection. J Comp Neurol 211:65-83.

Pickard GE (1985) Bifurcating axons of retinal ganglion cells terminate in the hypothalamic suprachiasmatic nucleus and the intergeniculate leaflet of the thalamus. Neurosci Lett 55:211-217.

Provencio I and Foster RG (1995) Circadian rhythms in mice can be regulated by photoreceptors with cone-like characteristics. Brain Res 694:183-190.

Rosenthal NE, Sack DA, Gillin JC, Lewy AJ, Goodwin FK, Davenport Y, Mueller PS, Newsome DA, and Wehr TA (1984) Seasonal affective disorder: A description of the syndrome and preliminary findings with light therapy. Arch Gen Psychiatr 41:72-80.

Tessonneaud A, Locatelli A, Caldani M, and Viguier Martinez MC (1995) Bilateral lesions of the suprachiasmatic nuclei alter the nocturnal melatonin secretion in sheep. J Neuroendocrinol 7:145-152.

Wise GN, Dollery CT, and Henkind P (1971) *The Retinal Circulation*, Harper & Row, New York.

Wright KP Jr., Badia P, Myers BL, Plenzler SC, and Drake CL (1997) Caffeine and bright light effects on nighttime melatonin and body temperature in women during sleep deprivation. Sleep Res 26:636.